

Claims:

1. A method for identifying a compound capable of modulating influx of calcium ions into a eukaryotic cell comprising:

- (a) providing a eukaryotic cell having a detectable reporter capable of translocation from the cytosol to associate with the plasma membrane in response to an influx of calcium ions,
- (b) incubating the cell with a test compound,
- (c) providing a stimulus for calcium influx, and,
- (d) monitoring association of the detectable reporter with the plasma membrane and/or a decrease in the detectable reporter in the cytosol,

wherein the stimulus for calcium influx is provided before during and/or after incubation of the cell with the test compound.

2. A method according to claim 1 comprising:

- (a) monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from cytosol while
 - (i) providing a stimulus for calcium influx by incubating the cell in conditions that stimulate calcium influx, then
 - (ii) providing a test compound and incubating the cell with the test compound, and,
- (b) comparing the association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol in the absence of the test compound with the association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol in the presence of the test compound,

wherein a difference in the association of the detectable reporter with the membrane and/or loss of detectable reporter from the cytosol in the presence of the test compound indicates that the test compound modulates calcium influx.

3. A method for according to claim 2, for identifying a compound that antagonises calcium ion influx comprising:

- (a) monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol while
 - (i) providing a stimulus for calcium influx by incubating the cell in conditions that stimulate calcium influx, then,
 - (ii) providing a test compound and incubating the cell with the test compound, and,
- (b) comparing the association of the detectable reporter with the membrane and/or loss of detectable reporter from the cytosol in the absence and presence of the test compound,

wherein a decrease in association of the detectable reporter with the membrane and/or an increase in the detectable reporter in the cytosol following incubation of the cell with the test compound indicates that the test compound antagonises calcium ion influx.

4. A method for according to claim 1 for identifying a compound that modulates calcium ion influx comprising:

- (a) providing a control by monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol while incubating the cell in conditions that stimulate calcium influx,
- (b) monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol while
 - (i) incubating the cell with a test compound, then
 - (ii) providing a stimulus for calcium influx by incubating the cell in conditions that stimulate calcium influx, and
- (c) comparing the association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol in the absence and presence of the test compound,

wherein a difference in association of the detectable reporter with the membrane and/or a difference in loss of the detectable reporter from the cytosol in the presence of the test compound compared to that in the absence of the test compound indicates that the test compound modulates calcium influx.

5. A method according to claim 4, for identifying a compound that antagonises calcium ion influx comprising:

- (a) providing a control by monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol while providing a stimulus for calcium influx by incubating the cell in conditions that stimulate calcium influx,
- (b) monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol while
 - (i) incubating the cell with a test compound, then
 - (ii) providing a stimulus for calcium influx by incubating the cell in conditions that stimulate calcium influx, and
- (c) comparing the association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol in the absence and presence of the test compound,

wherein a decrease in the association of the detectable reporter with the membrane and/or a decrease in loss of the detectable reporter from the cytosol in the presence of the test compound compared to that in the absence of the test compound indicates that the test compound antagonises calcium influx.

6. A method according to claim 1, for identifying a compound that agonises calcium ion influx comprising:

- (a) providing a eukaryotic cell having a detectable reporter capable of translocation from the cytosol to associate with the plasma membrane in response to an influx of calcium ions,
- (b) providing a control by monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol while incubating the cell in conditions that stimulate calcium influx,
- (c) monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol while
 - (i) providing a test compound and incubating the cell with the test compound, then,

- (ii) providing a stimulus for calcium influx by incubating the cell in conditions that stimulate calcium influx, and,
- (d) comparing the association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol in the absence and presence of the test compound,

wherein an increase in the association of the detectable reporter with the membrane and/or an increase in loss of detectable reporter from the cytosol in the presence of the test compound compared to that in the absence of the test compound indicates that the test compound agonises calcium influx.

7. A method for identifying a compound that agonises calcium ion influx comprising:

- (a) providing a eukaryotic cell having a detectable reporter capable of translocation from the cytosol to associate with the plasma membrane in response to an influx of calcium ions,
- (b) monitoring association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol before and after incubation of the cell with the test compound, and,
- (c) comparing the association of the detectable reporter with the membrane and/or loss of the detectable reporter from the cytosol in the absence and presence of the test compound,

wherein an increase in association of the detectable reporter with the membrane and/or a decrease in the detectable reporter in the cytosol following introduction of the test compound indicates that the test compound agonises calcium ion influx.

8. A method according to any preceding claim wherein the cell is a mammalian cell.

9. A method according to any preceding claim wherein the cell is a CHO, Cos, Jurkat-T, HeLa, PC12, HEK293, J774 mouse macrophage, B6MP102 mouse macrophage, THP-1 monocyte-macrophage, RAW264.7 macrophage,

HL-60 myeloid precursor, PLB-986 promyelocytic, Jurkat, A20, Raji, T-lymphocyte or B-lymphocyte cell.

10. A method according to any preceding claim wherein the cell is capable of expressing, endogenously or ectopically, one or more calcium channel selected from the group comprising: TRP family channels, voltage-gated channels, ligand-gated channels and receptor-operated channels.

11. A method according to any preceding claim wherein the detectable reporter is expressed within the eukaryotic cell.

12. A method according claim 11 wherein a nucleic acid encoding the detectable reporter is stably integrated within the eukaryotic cell.

13. A method according to any one of claims 1 to 11 wherein a nucleic acid encoding the detectable reporter is transiently transfected into the eukaryotic cell.

14. A method according to any one of claims 1 to 10 wherein the detectable reporter is introduced into the cell.

15. A method according to any preceding claim wherein the detectable reporter is or comprises CAPRI, or a derivative thereof which is capable of translocation to and association with the plasma membrane, and a detectable marker.

16. A method according to any preceding claim wherein the detectable reporter is or comprises the CAPRI derivative R473S, and a detectable marker.

17. A method according to any preceding claim wherein the detectable reporter is a CAPRI derivative comprising or consisting of the CAPRI C2A C2B domains, with wild type or mutated sequence, and a detectable marker.

18. A method according to any preceding claim wherein the detectable reporter is a reporter labelled with a fluorescent marker.

19. A method for identifying a compound that modulates calcium ion influx comprising:

- (a) providing a eukaryotic cell expressing FP-CAPRI or a FP-CAPRI derivative which is capable of translocation to and association with the plasma membrane;
- (b) (i) as a control, incubating the eukaryotic cell in the absence of test compound then providing a stimulus for calcium influx, and,
(ii) incubating the eukaryotic cell in the presence of a test compound, then providing a stimulus for calcium influx; or;
- (c) (i) as a control, incubating the eukaryotic cell and providing a stimulus for calcium influx in the absence of test compound, and,
(ii) incubating the eukaryotic cell and providing a stimulus for calcium influx, then providing a test compound;
and,
- (d) monitoring fluorescence of the cell cytosol and/or plasma membrane during incubation in (b) or (c),

wherein a difference in fluorescence in the cytosol and/or at the plasma membrane in the presence of the test compound compared with that in the absence of the test compound is indicative that the test compound modulates calcium ion influx.

20. A method for identifying a compound that agonises calcium ion influx comprising:

- (a) providing a eukaryotic cell expressing FP-CAPRI or a FP-CAPRI derivative, which is capable of translocation to and association with the plasma membrane,
- (b) incubating the eukaryotic cell in the presence of a test compound,
and,
- (c) monitoring fluorescence of the cell cytosol, and/or plasma membrane,

wherein a decrease in cytosolic fluorescence, and/or increase in plasma membrane fluorescence following addition of the test compound is indicative that the test compound agonises calcium ion influx.

21. A method for identifying a compound that antagonises calcium ion influx comprising:

- (a) providing a eukaryotic cell expressing FP-CAPRI or a FP-CAPRI derivative which is capable of translocation to and association with the plasma membrane,
- (b) incubating the eukaryotic cell and providing a stimulus for calcium ion influx,
- (c) monitoring fluorescence of the cell cytosol and/or plasma membrane,
- (d) introducing a test compound to the incubation mixture,
- (e) monitoring fluorescence of the cell cytosol and/or plasma membrane,

wherein an increase in cytosolic fluorescence and/or a decrease in plasma membrane fluorescence following addition of the test compound is indicative that the test compound antagonises calcium ion influx.

22. A method for identifying a compound that agonises calcium ion influx comprising:

- (a) providing a eukaryotic cell expressing FP-CAPRI or a FP-CAPRI derivative which is capable of translocation to and association with the plasma membrane,
- (b) incubating the eukaryotic cell in the presence of a test compound and,
- (c) monitoring fluorescence of the cell cytosol and/or plasma membrane,

wherein a decrease in cytosolic fluorescence and/or an increase in plasma membrane fluorescence following addition of the test compound is indicative that the test compound agonises calcium ion influx.

23. A method according to any one of claims 18 to 22, wherein the fluorescent marker is a quantum dot.

24. A method according to any one of claims 18 to 22, wherein the fluorescent marker is a fluorescent protein.

25. A method according to claim 24, wherein the fluorescent protein is a red, orange, yellow, yellow-green, green-yellow, green or blue (cyan) fluorescent protein.

26. A method according to claim 24, wherein the fluorescent protein is a green fluorescent protein (GFP).

27. A method according to any one of claims 24 to 26, wherein the fluorescent protein is a wild type, enhanced, destabilised enhanced, or red-shift fluorescent protein.

28. A method according to any one of claims 18 to 27, wherein monitoring is performed by fluorescence microscopy.

29. A method according to claim 28, wherein fluorescence microscopy is performed by wide-field or total internal reflection fluorescence microscopy, fluorescence lifetime imaging or confocal imaging.

30. A method according to any one of claims 18 to 29, wherein monitoring is performed by measuring fluorescence at the region(s) of interest within the cell over time.

31. A method according to claim 30, wherein monitoring association of the detectable reporter with the plasma membrane and/or decrease of detectable reporter in the cytosol is assessed by calculating the relative translocation parameter $(1-F_t/F_o)$ at one or more time points, wherein F_o is the fluorescence in a region(s) of interest at the start of monitoring and F_t is fluorescence in a region(s) of interest at a later time point or points.

32. A method according to claim 31, wherein the region(s) of interest is the cytosol and/or plasma membrane.

33. A method according to claim 31 or claim 32, wherein monitoring is performed by measuring cytosolic fluorescence over time and calculating the relative translocation parameter at one or more time points, $1 - F_{t_{\text{cyt}}} / F_{o_{\text{cyt}}}$, wherein $F_{o_{\text{cyt}}}$ is the cytosolic fluorescence in the region of interest at the start of monitoring and $F_{t_{\text{cyt}}}$ is the cytosolic fluorescence in the region of interest at a particular time point.

34. A method according to any preceding claim performed in high throughput format.

35. A method according to any preceding claim wherein the test compound is not ATP or histamine.

36. A method according to any preceding claim wherein prior to incubation of the cell with the test compound and/or provision of the stimulus for calcium influx, the cell is incubated in calcium ion depleted medium with a compound that is capable of depleting the intracellular calcium store.

37. A method according to claim 36, wherein the compound capable of depleting the intracellular calcium store cell is thapsigargin.

38. A method according to claims 36 or 37, wherein calcium ions are added to the medium prior to, during and/or after provision of the test compound to the cell.